Appendix A

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Aerosol cyclosporin therapy in lung transplant recipients with bronchiolitis obliterans

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ABSTRACT: The majority of patients who develop bronchiolitis obliterans, after lung transplantation, die within 2–3 yrs after onset since treatment with conventional immunosuppression is typically ineffective. A case/control study was conducted in lung transplant recipients with biopsy-documented bronchiolitis obliterans to determine whether aerosol cyclosporin use contributed to increased survival.

The cases comprised 39 transplant recipients who received open-label aerosol cyclosporin treatment in addition to conventional immunosuppression. The controls were transplant recipients treated with conventional immunosuppression alone. There were 51 controls from the University of Pittsburgh Medical Center and 100 from a large multicentric database (Novartis Lung Transplant Database).

Forced expiratory volume in one second expressed as a percentage of the predicted value was an independent predictor of survival in all patients with bronchiolitis obliterans. Cox proportional-hazards analysis revealed a survival advantage for aerosol cyclosporin cases compared to the Pittsburgh control group. A survival advantage was also seen when comparing study cases to multicentric controls.

Aerosol cyclosporin, given with conventional immunosuppression to lung transplant recipients with bronchiolitis obliterans, provides a survival advantage over conventional therapy alone.

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The 3-yr survival after lung transplantation is 55% [1]. More than half of all lung transplant recipients who survive ⩾1 yr develop chronic allograft rejection that is associated with bronchiolitis obliterans [2–5]. The development of bronchiolitis obliterans has a negative impact on long-term survival. No immunosuppressive regimen has been shown to prevent this complication or improve survival once it occurs [6–8].

Bronchiolitis obliterans is thought to be a consequence of immunological lung injury [9–11]. An inflammatory cascade directed against allograft bronchiolar epithelial and endothelial cells is initiated by T-lymphocytes, as demonstrated in animals [12–14] and human subjects [15, 16]. Alloreactive lymphocytes are found in bronchoalveolar lavage fluid, and selective T-cell clones are expanded during bronchiolitis obliterans [17]. Myriad cytokines and lymphocyte gene expression induce small airway inflammation, leading to myofibroblast-like cell proliferation and ingrowth of small airway granulation tissue, and resulting in occlusion of the airway lumen [18–21]. A cascade of injury and remodelling triggers a fibroproliferative process responsible for bronchiolar scarring and graft failure.

Cyclosporin is one of the mainstays of maintenance immunosuppression after transplantation; however, it has a narrow therapeutic index when given systemically [22]. It blocks lymphocyte activation by inhibiting transcription of cytokine genes in a dose-dependent manner [22, 23]. Cyclosporin also exerts an antifibrotic effect by inhibiting alveolar macrophage function and suppressing collagen deposition by lung fibroblasts [24, 25].

Previous investigations in animal lung transplant models demonstrated that aerosol cyclosporin produces higher concentrations of cyclosporin in lung tissue than does systemic administration and also decreases allograft inflammation associated with rejection [26–30]. The present authors have used aerosol cyclosporin to treat lung recipients with refractory rejection since 1991 [31]. Delivery of cyclosporin directly into the rejecting lung ameliorates small airway inflammation associated with bronchiolitis obliterans [32, 33]. In addition, pro-inflammatory cytokine gene expression in bronchoalveolar lavage cells decreases during treatment [34–36]. However, the therapeutic effect of aerosol cyclosporin is dependent on the dose deposited in the transplanted lung [36]. The present study demonstrates that aerosol cyclosporin also reduces the risk of death in patients with bronchiolitis obliterans.

Materials and methods

Lung transplant recipients with bronchiolitis obliterans

The present study was conducted at the University of Pittsburgh Medical Center, Pittsburgh, PA, USA. A total of

538 lung transplant recipients from the Pittsburgh Lung Transplant Registry were screened and 129 were eligible for the study. The portion of this group that received open-label rescue treatment with aerosol cyclosporin constituted the study cases. The remainder, who did not meet entry criteria, were eligible to serve as controls (Pittsburgh control group) (fig. 1). A second control group was obtained from the Novartis Lung Transplant Database, Stanford, CA, USA (n=100) (fig. 2). This registry includes 826 lung transplant recipients from 12 major lung transplant centres throughout the world [37]. The study cases and both control groups underwent lung transplantation in the period January 1991–March 2001 and were required to have biopsy-proven bronchiolitis obliterans. Identical criteria were used to select the Pittsburgh and multicentric control group patients, all of whom received conventional immunosuppression alone. Survival after bronchiolitis obliterans in the study cases was compared to the two distinct contemporaneous control groups.

Variables affecting survival after bronchiolitis obliterans

Variables potentially affecting survival after bronchiolitis obliterans were analysed in transplant recipients from the Pittsburgh Lung Transplant Registry (n=129). Recipients were included if they met the following criteria: 1) receipt of a single lung, double lung or heart/lung transplant; 2) transplantation in the period January 1991–March 2001; 3) histological diagnosis of bronchiolitis obliterans; and 4) no retransplantation. The histological diagnosis of lung transplant rejection was made according to standard criteria [38]. A total

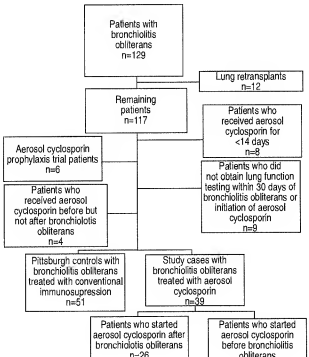

Fig. 1.–Selection criteria for lung transplant recipients from the Pittsburgh Lung Transplant Registry who underwent lung transplantation in the period January 1991–March 2001 and had biopsy-proven bronchiolitis obliterans (: excluded patients). Cases who received aerosol cyclosporin were compared to Pittsburgh controls treated with conventional immunosuppression alone.

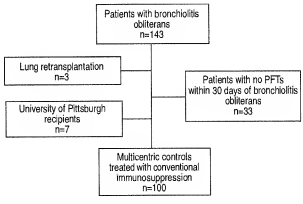

Fig. 2.–Selection criteria for lung transplant recipients from the Novartis Lung Transplant Database multicentric control group who underwent lung transplantation in the period January 1991–March 2001 and had biopsy-proven bronchiolitis obliterans (: excluded patients). PFT: pulmonary function test.

of 117 recipients met these criteria (fig. 1) (table 1). Ten variables were examined as possible predictors of survival (table 2). The determination of factors predictive of survival was performed using first univariate and then multivariate Cox proportional-hazards modelling.

Aerosol cyclosporin patient selection criteria

The Institutional Review Board of the University of Pittsburgh approved aerosol cyclosporin as open-label rescue treatment for refractory allograft rejection. In order to be eligible to receive aerosol cyclosporin, patients were required to experience refractory rejection. Refractory rejection was

Table 1.–Baseline characteristics of lung transplant recipients with bronchiolitis obliterans (BO) from the Pittsburgh Lung Transplant Registry used to analyse possible predictors of survival

Females/males n	69/48
Indication for transplantation	
Emphysema	44 (38)
Cystic fibrosis	20 (17)
Fibrotic lung disease	17 (15)
Primary pulmonary hypertension	10 (8)
Other	26 (22)
Type of transplant	
Single lung	60 (51)
Double lung	47 (40)
Heart/lung	10 (9)
Bronchiolitis obliterans	
Age at diagnosis yrs	44.4±13.2
Time after transplant of diagnosis days	727±639; 524 (318–936)
FEV1[#] % pred	53±23
Acute rejection[*] events before BO	3.9±2.5; 4 (2–5)

Data are presented as n (%), mean±SD or median (interquartile range). FEV1: forced expiratory volume in one second; % pred: per cent predicted. [#]: measured at initiation of aerosol cyclosporin (AC) therapy (cases who received AC only after histological confirmation of BO) or at time of histological diagnosis of BO (controls and all other cases); [*]: at least grade 2 acute cellular rejection, *i.e.* mild rejection typically treated with pulsed methylprednisolone or antithymocyte globulin.

Table 2. – Univariate and multivariate analysis of factors affecting survival after bronchiolitis obliterans (BO)

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
FEV1[#] % pred	0.98 (0.96–0.99)	<0.001	0.97 (0.96–0.99)	<0.001
Lung transplantation date				
1991–1993 *versus* 1994–1996	1.12 (0.62–2.03)	0.70	1.08 (0.58–2.03)	0.81
1991–1993 *versus* 1997–2001	1.21 (0.46–3.14)	0.70	1.10 (0.38–3.18)	0.86
Time after transplant of BO diagnosis days	1.00 (1.00–1.00)	0.54	1.00 (0.99–1.00)	0.63
Transplant type				
Double *versus* single	1.31 (0.80–2.16)	0,29	1.62 (0.95–2.77)	0.08
Heart/lung *versus* single	1.67 (0.73–3.81)	0.22	2.40 (1.04–5.56)	0.04
Blood creatinine level at BO diagnosis	1.03 (0.81–1.33)	0.77	1.05 (0.81–1.36)	0.72
Female sex	0.77 (0.48–1.24)	0.29	0.85 (0.51–1.41)	0.53
Age at BO diagnosis yrs	1.00 (0.98–1.02)	0.90	1.00 (0.98–1.02)	0.81
Acute rejection[¶] events before BO n	0.98 (0.89–1.08)	0.76	0.96 (0.87–1.05)	0.36
Acute rejection[¶] events·pre-BO biopsy^{-1}[+]	0.72 (0.23–2.21)	0.57	0.71 (0.22–2.30)	0.57
Acute rejection[¶] events·pre-BO yr^{-1}[+]	1.01 (0.95–1.07)	0.67	1.00 (0.93–1.06)	0.91

HR: hazard ratio; CI: confidence interval; FEV1: forced expiratory volume in one second; % pred: per cent predicted. [#]: measured at initiation of aerosol cyclosporin (AC) therapy (cases who received AC only after histological confirmation of BO) or at time of histological diagnosis of BO (controls and all other cases); [¶]: at least grade 2 acute cellular rejection, *i.e.* mild rejection typically treated with pulsed methylprednisolone or antithymocyte globulin; [+]: ratio of acute rejection events to routine surveillance biopsy procedures before BO diagnosis, a measure of high-grade acute rejection frequency

defined according to the following three criteria: 1) two or more consecutive histological rejection events (active bronchiolitis obliterans, at least grade 2 acute rejection and/or lymphocytic bronchitis/bronchiolitis); 2) ⩾10% decline in forced expiratory volume in one second (FEV1) expressed as a percentage of the predicted value from the best baseline value; and 3) failure to improve histologically following conventional immunosupressive therapy. Of the 129 recipients with bronchiolitis obliterans from the Pittsburgh Lung Transplant Registry, those who received aerosol cyclosporin were the study cases (n=39) (fig. 1). Study cases were included if they met the following criteria: 1) receipt of aerosol cyclosporin for ⩾14 days; 2) receipt of aerosol cyclosporin after histological confirmation of bronchiolitis obliterans if treatment commenced prior to this diagnosis; 3) pulmonary function tests carried out within 30 days of either the diagnosis of bronchiolitis obliterans or initiation of aerosol cyclosporin; and 4) nonparticipation in other aerosol cyclosporin protocols. Of the 39 study cases, 26 received aerosolised cyclosporin only after histological confirmation of bronchiolitis obliterans and 13 prior to confirmation of bronchiolitis obliterans. Only four subjects who began treatments with aerosol cyclosporin were unable to continue with treatment for ⩾2 weeks due to cough, dyspnoea and other symptoms of upper airway irritation.

Administration of aerosol cyclosporin

Aerosol cyclosporin was delivered from a jet nebuliser (AeroTech II; Cis-Us, Bedford, MA, USA) containing 300 mg drug daily for 10 days followed by 300 mg three times per week, on Mondays, Wednesdays and Fridays [8]. Cyclosporin powder (Novartis Pharmaceuticals, East Hanover, NJ, USA) was dissolved in propylene glycol at a concentration of 62.5 mg·mL^{-1}.

Immunosuppressive regimens

Maintenance immunosuppression consisted of oral cyclosporin or tacrolimus, azathioprine or mycophenolate mofetil, and prednisone. Enhanced immune suppression for treatment of acute rejection (at least grade 2) and/or active bronchiolitis obliterans consisted of pulse corticosteroids (intravenous methylprednisolone, 1 g·day^{-1} for 3 days, or oral prednisone, 100 mg tapered to 10 mg over 14 days), equine antilymphocyte globulin (Atgam; Pharmacia, Kalamazoo, MI, USA; 15 mg·kg body weight^{-1}·day^{-1} for 7 days) or rabbit antithymocyte globulin (Thymoglobulin; Sangstat, Fremont, CA, USA; 1.5 mg·kg body weight^{-1}·day^{-1} for 5–7 days).

Statistical considerations in determining the effect of aerosol cyclosporin on survival

The primary goal was to determine whether or not aerosol cyclosporin improved survival after diagnosis of bronchiolitis obliterans. Overall survival time was determined from the initiation of aerosol cyclosporin in the 26 patients with confirmed bronchiolitis obliterans prior to receiving aerosol cyclosporin, and on the day of histological confirmation of bronchiolitis obliterans in the 13 patients who received aerosol cyclosporin prior to diagnosis of bronchiolitis obliterans. In both control groups, survival time was determined from the date bronchiolitis obliterans was confirmed. Baseline FEV1 (as a percentage of the predicted value) and bronchiolitis obliterans syndrome grades for each subject were similarly determined on the date of either aerosol cyclosporin initiation or confirmation of bronchiolitis obliterans, using the criteria described above. Overall survival was assessed in the 39 aerosol cyclosporin cases and the 51 Pittsburgh and 100 multicentric control patients using the Kaplan-Meier method, and comparisons were made using the log-rank test [39].

Cox proportional-hazards modelling was then used to account for potential confounders in the analysis, and to generate a final asscssment of the effect of aerosol cyclosporin on survival. Factors determined to be significant predictors of survival in the Pittsburgh Lung Transplant Registry group were included in the model, as was a variable intended to gauge the extent of refractory rejection, the condition that originally resulted in the cases receiving aerosol cyclosporin. It was decided *a priori* that the number of at least grade 2

rejection events per year prior to diagnosis of bronchiolitis obliterans would best serve this function. Analyses were performed in all 39 aerosol cyclosporin study cases and in the subgroup of 26 cases who received aerosol cyclosporin after histological confirmation of bronchiolitis obliterans. (These study cases were compared independently to both the Pittsburgh and multicentric control groups.) Estimates of the hazard ratio with 95% confidence intervals were calculated [40].

Results

Characteristics of the patients

The study population comprised 39 cases and 151 controls (table 3). Aerosol cyclosporin was administered for a mean duration of 69.9±66.3 weeks. Twenty-five (64.1%) cases demonstrated a reduction in their grade of rejection by transbronchial biopsy within 90 days after initiation of aerosol cyclosporin treatment. The baseline FEV1 was lower in the aerosol cyclosporin cases than in the Pittsburgh and multicentric controls (45±16 *versus* 56±26 and 53±21% pred (p=0.03 and p=0.04), respectively). Acute rejection was more common prior to the onset of bronchiolitis obliterans in study cases compared to Pittsburgh and multicentric controls (4.8 *versus* 3.2 and 1.4 at least grade 2 rejection events, respectively). Bronchiolitis obliterans syndrome, at baseline, was more prevalent in the aerosol cyclosporin group than in the Pittsburgh controls (79 *versus* 55%, p=0.02 by Chi-squared test), and similar in proportion to that in the multicentric controls (70%, p=0.26). The number of cytomegalovirus mismatches (donor negative/recipient positive) was similar in cases *versus* Pittsburgh controls (9 *versus* 12, p=0.15). There was no difference in cytomegalovirus disease after bronchiolitis obliterans or aerosol cyclosporin, based on bronchoalveolar lavage culture or histology in cases and Pittsburgh controls (four cases identified histologically in both groups). Bronchiolitis obliterans was detected earlier after transplantation in aerosol cyclosporin cases compared to the Pittsburgh controls. However, this difference was nonsignificant (688±524 *versus* 844±686 days after transplant, p=0.28). Baseline immunosuppression and antiviral prophylaxis were similar in the study and control cases in terms of their use of the basic medications and their dosing, and mean tacrolimus and cyclosporin levels after bronchiolitis obliterans diagnosis were similar in cases and Pittsburgh controls (14.1±3.5 *versus* 13.6±4.3; 318.9±198.2 *versus* 322.4±92.8) (table 4).

Table 4. – Comparison of baseline immunosuppression and antiviral prophylaxis between aerosol cyclosporin cases and Pittsburgh controls

Aerosol cyclosporin cases *versus* Pittsburgh controls	p-value[#]
Medication use	
Tacrolimus and oral cyclosporin	0.11
Azathioprine	0.48
Mycophenolate mofetil	0.25
Aciclovir	0.37
Ganciclovir	0.15
Valganciclovir	0.44
Dose	
Tacrolimus	0.93
Oral cyclosporin	0.59
Oral prednisone	0.65
Azathioprine	0.27

[#]: Chi-squared test for proportion of subjects using medications and unpaired t-test for dose on day of bronchiolitis obliterans diagnosis.

Table 3. – Baseline characteristics of aerosol cyclosporin (AC) cases and Pittsburgh and multicentric control groups

	Cases		Controls	
	Total	AC after BO[#]	Pittsburgh	Multicentric
Females/males n	24/15	18/8	31/20	46/54
Indication for transplantation				
Emphysema	9 (23)	4 (15)	22 (43)	36 (36)
Cystic fibrosis	10 (26)	8 (31)	6 (11)	20 (20)
Fibrotic lung disease	11 (28)	7 (27)	5 (10)	13 (13)
Primary pulmonary hypertension	2 (5)	1 (4)	8 (16)	14 (14)
Other	7 (18)	6 (23)	10 (20)	17 (17)
Type of transplant				
Single lung	19 (49)	12 (46)	30 (59)	50 (50)
Double lung	18 (46)	13 (50)	14 (27)	25 (25)
Heart/lung	2 (5)	1 (4)	7 (14)	25 (25)
Bronchiolitis obliterans				
Age at diagnosis yrs	40.6±13.3	38.8±13.1	45.1±13.1	43.3±13.2
Time after transplant of diagnosis days	688±524; 557 (321–820)	519±388; 460 (260–646)	844±686; 585 (348–1106)	616±379; 544 (315–901)
FEV1[+] % pred	45±16	47±16	56±26	53±21
Acute rejection events[¶] before BO	4.8±3.0; 4 (4–7)	3.8±2.2; 3 (2–6)	3.2±1.9; 3 (2–4)	1.4±1.5; 1 (0–2)
Grade 3 BOS[+]	8 (21)	4 (15)	8 (15)	21 (21)
Grade 2 BOS[+]	8 (21)	6 (23)	10 (20)	18 (18)
Grade 1 BOS[+]	15 (37)	10 (39)	10 (20)	31 (31)
No BOS[+]	8 (21)	6 (23)	23 (45)	30 (30)
AC therapy duration weeks	69.9±66.3	71.5±0.7	NA	NA
Time between diagnosis and AC therapy weeks	NA	36.4±36.1	NA	NA

Data are presented as n (%), mean±SD or median (interquartile range). BO: bronchiolitis obliterans; FEV1: forced expiratory volume in one second; BOS: bronchiolitis obliterans syndrome [41]; % pred: per cent predicted; NA: not applicable. [#]: AC received only after histological confirmation of BO; [¶]: at least grade 2 acute cellular rejection, *i.e.* mild rejection typically treated with pulsed methylprednisolone or antithymocyte globulin; [+]: measured at initiation of AC therapy (cases who received AC only after histological confirmation of BO) or at time of histological diagnosis of BO (controls and all other cases).

Kaplan-Meier survival analysis

Kaplan-Meier estimates of survival are shown in figure 3. Median survival was 4.5 yrs in aerosol cyclosporin cases (n=39), 2.4 yrs in Pittsburgh controls and 2.3 yrs in multicentric controls. Median survival in the 26 cases who started aerosol cyclosporin therapy after bronchiolitis obliterans diagnosis was 4.5 yrs.

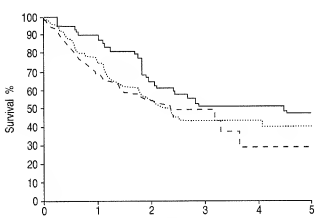

Fig. 3. – Kaplan-Meier survival estimates for aerosol cyclosporin (AC) cases (—; n=39) and the Pittsburgh (·····; n=51) and multicentric (- - - -; n=100) control groups. Survival was estimated from the initiation of AC therapy (cases who received AC only after histological confirmation of bronchiolitis obliterans (BO)) or time of histological diagnosis of BO (controls and all other cases). The p-values obtained at 1, 2, 3, 4 and 5 yrs were 0.11, 0.21, 0.23, 0.23 and 0.26 for the Pittsburgh controls and 0.02, 0.12, 0.22, 0.08 and 0.09 for the multicentric controls, respectively (log-rank test).

Proportional-hazards modelling of prognostic factors

Factors potentially predictive of survival after bronchiolitis obliterans diagnosis were analysed in all 117 patients from the Pittsburgh Lung Transplant Registry (table 2). Preservation of FEV1 (as a percentage of the predicted value) was associated with a reduced risk of death (hazard ratio 0.98, p<0.001). When FEV1 was included in a multivariate model, a trend towards better survival was noted in single lung *versus* heart/lung (hazard ratio 2.40, p=0.04) and double lung (hazard ratio 1.62, p=0.08) recipients. Importantly, no significant differences in survival risk were noted between date of transplantation intervals (1991–1993, 1994–1996 and 1997–2001) or the time after transplant (in days) of bronchiolitis obliterans diagnosis (table 2).

Multivariate survival analysis

Table 5 shows the results of the Cox proportional-hazards model of the effect of aerosol cyclosporin on survival after a histological diagnosis of bronchiolitis obliterans. Aerosol cyclosporin was associated with improved survival compared to the Pittsburgh controls (hazard ratio 0.48, p=0.03). A similar survival advantage, approaching significance, was evident in the subgroup of 26 cases who received aerosol cyclosporin only after diagnosis of bronchiolitis obliterans (hazard ratio 0.49, p=0.06). Survival benefit was also demonstrated for the entire study group and the subgroup compared to multicentric controls (hazard ratio 0.40 (p=0.01) and 0.41 (p=0.03), respectively). The cyclosporin cases demonstrated a similar advantage when compared to the combined control group (hazard ratio 0.46, p=0.01).

Table 5. – Results of Cox proportional-hazards model considering survival after a histological diagnosis of bronchiolitis obliterans (BO) in both aerosol cyclosporin (AC) cases and controls

Cases	Controls		Hazard ratio (95% CI)	p-value
AC after BO#	Pittsburgh	Aerosol cyclosporin	0.49 (0.23–1.03)	0.06
		FEV1+ % pred	0.97 (0.96–0.99)	0.001
		Acute rejection¶ events·yr^{-1}	0.95 (0.85–1.05)	0.31
		Transplant type		
		Double *versus* single	2.01 (0.99–4.09)	0.05
		Heart/lung *versus* single	2.28 (0.84–6.17)	0.10
	Multicentric	Aerosol cyclosporin	0.41 (0.19–0.89)	0.03
		FEV1+ % pred	0.96 (0.94–0.98)	<0.001
		Acute rejection¶ events·yr^{-1}	0.98 (0.90–1.08)	0.75
		Transplant type		
		Double *versus* single	1.24 (0.69–2.23)	0.48
		Heart/lung *versus* single	1.11 (0.51–2.45)	0.79
Total	Pittsburgh	Aerosol cyclosporin	0.48 (0.25–0.93)	0.03
		FEV1+ % pred	0.97 (0.96–0.99)	<0.001
		Acute rejection¶ events·yr^{-1}	0.96 (0.87–1.06)	0.40
		Transplant type		
		Double *versus* single	1.85 (0.96–3.57)	0.07
		Heart/lung *versus* single	1.54 (0.81–5.82)	0.12
	Multicentric	Aerosol cyclosporin	0.40 (0.20–0.77)	0.01
		FEV1+ % pred	0.96 (0.94–0.98)	<0.001
		Acute rejection¶ events·yr^{-1}	0.99 (0.91–1.08)	0.88
		Transplant type		
		Double *versus* single	1.19 (0.86–2.09)	0.54
		Heart/lung *versus* single	1.12 (0.51–2.44)	0.78

FEV1: forced expiratory volume in one second; pred: per cent predicted. #: AC received only after histological confirmation of BO; ¶: at least grade 2 acute cellular rejection, *i.e.* mild rejection typically treated with pulsed methylprednisolone or antithymocyte globulin; +: measured at initiation of AC therapy (cases who received AC only after histological confirmation of BO) or at time of histological diagnosis of BO (controls and all other cases).

Discussion

The present results indicate that aerosol cyclosporin improves survival among lung transplant recipients with bronchiolitis obliterans. Only patients with biopsy-proven bronchiolitis obliterans were included since a precise assessment of survival time was dependent on the specificity of this diagnosis. Median survival was ~2 yrs longer in recipients who received aerosol cyclosporin than in the Pittsburgh and multicentric controls, and a trend approaching significance was demonstrated using the log-rank test (4.5 *versus* 2.4 and 2.3 yrs ($p=0.21$ and $p=0.09$), respectively). In support of the reliability of these data, the median survival in both control populations was very similar, and almost identical to the published median survival of lung transplant recipients with bronchiolitis obliterans syndrome from the Barnes-Jewish Hospital in St Louis (MO, USA) [9], a centre with one of the largest experiences in lung transplantation in the USA.

Covariates that might influence survival after bronchiolitis obliterans were analysed in 117 patients with biopsy-proven bronchiolitis obliterans chosen from the Pittsburgh Lung Transplant Registry. The degree of lung function impairment was a significant predictor of mortality. Heart/lung and double lung transplant recipients were at increased risk of death after the onset of bronchiolitis obliterans compared to single lung transplant recipients. A multivariate model was utilised in order to determine the effect of aerosol cyclosporin on survival. Variables shown to predict survival after a diagnosis of bronchiolitis obliterans were included as potential confounders, as was a variable gauging the extent of refractory rejection, the primary enrolment condition for the original open-label study of aerosol cyclosporin (though not an independent predictor of survival after bronchiolitis obliterans based on the current model). The model indicates that the risk of death among all cases who received aerosol cyclosporin was reduced by ~50% compared to the Pittsburgh controls. A similar reduction in fatal outcomes was observed in the subgroup of 26 patients who received aerosol cyclosporin only after confirmation of bronchiolitis obliterans. Furthermore, a comparable reduction in the risk of death was evident when the control group was derived exclusively from the Novartis Lung Transplant Database.

Survival after bronchiolitis obliterans correlates with the extent of functional deterioration of the lung allograft. FEV1, expressed as a percentage of the predicted value, appears to represent a reliable physiological marker of the morphological extent of small airway pathology, with successful management being dependent on the degree of reversible active airway inflammation as opposed to bronchiolar fibrosis. In addition, single lung recipients demonstrated a survival benefit compared to heart/lung and double lung transplant recipients. The native lung, under the circumstances of bronchiolitis obliterans, could offer a protective effect since functional deterioration in the native lung is slow compared to the relatively rapid deterioration of the allograft associated with bronchiolitis obliterans. The native lung may also benefit from the systemic immunosuppressive effects of systemic cyclosporin or tacrolimus in patients transplanted for immunologically mediated lung disease [42].

The present study assessed the association between the clinical use of aerosol cyclosporin and survival using a prospectively defined hypothesis and methods of data collection. Conducting such a trial at a single transplant centre was necessary since a multicentric trial was not logistically feasible, lung transplant programmes typically do not have experience in aerosol drug development and the dry-powder cyclosporin necessary to constitute aerosol cyclosporin was available in limited supply. The duration of the trial and follow-up were consequently very long (10 yrs). As it is recognised that refractory lung transplant rejection is associated with very poor outcomes, patient selection was not randomised and open-label therapy was given. Given the inherent imperfections of such a study design, substantial effort was made to ensure a proper comparison between cases and controls. This included the use of multivariate models along with the careful inclusion of matching factors, as previously described, and the use of two contemporary and independent control groups.

In summary, the present results support the administration of aerosol cyclosporin to lung transplant patients with bronchiolitis obliterans. Owing to the recognised limitations of a case/control study, future randomised studies should be performed using aerosol cyclosporin for bronchiolitis obliterans, and incorporating incremental drug dosing and measurement of allograft drug deposition to formalise a dose/response relationship and optimise patient benefit [36, 43, 44].

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